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(71) Applicant *(for all designated States except US)*: **LIPID SCIENCES, INC.** [US/US]; 7068 Koll Center Parkway, Suite 401, Pleasanton, CA 94566 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **CHAM, Bill, E.** [NL/AU]; Villa Del Aripo, 353 Woodsland Drive, Sheldon, Queensland 4157 (AU).

(74) Agents: **MCDONALD, John, K.** et al.; Kilpatrick Stockton LLP, Suite 2800, 1100 Peachtree Street, Atlanta, GA 30309 (US).

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(54) Title: A METHOD OF TREATING AND PREVENTING INFECTIOUS DISEASES

(57) Abstract: The present invention relates to a method for reducing the occurrence and severity of infectious diseases, especially infectious diseases in which lipid-containing infectious organisms are found in biological fluids, such as blood. The present invention employs solvents useful for extracting lipids from the lipid-containing infectious organism, thereby reducing the infectivity of the infectious organism. The present invention also provides a vaccine composition, comprising a lipid-containing infectious organism, treated with solvents to reduce the lipid content of the infectious organism, combined with a pharmaceutically acceptable carrier. The vaccine composition is administered to an animal or a human to provide protection against the lipid-containing infectious organism.

A METHOD OF TREATING AND PREVENTING INFECTIOUS DISEASES

FIELD OF THE INVENTION

The present invention relates to a method for reducing the occurrence and severity of infectious diseases, especially infectious diseases in which infectious organisms are found in biological fluids, such as blood. The method of the present invention employs a system to treat infectious organisms which contain lipids. The present invention employs a solvent system useful for extracting lipids from the infectious organism, thereby reducing the infectivity of the infectious organism. The present invention also reduces the spread of infectious disease by providing a composition comprising a vaccine, comprising an infectious organism, treated with the method of the present invention to reduce the lipid content of the infectious organism, combined with a pharmaceutically acceptable carrier, and administered to an animal or a human.

BACKGROUND OF THE INVENTION

Infectious disease is a major cause of suffering and death throughout the world. Infectious disease of varied etiology affects billions of animals and humans each year and inflicts an enormous economic burden on society. Many infectious organisms contain lipid as a major component of the membrane that surrounds them. Organisms which produce infectious disease and contain lipid in their cell wall or envelope include but are not limited to bacteria, viruses, protozoa, molds, and fungi. Numerous bacteria and viruses which affect animals and humans cause extreme suffering, morbidity and mortality. Many bacteria and viruses travel throughout the body in biological fluids, such as blood. These and

other infectious organisms may be found in other biological fluids such as peritoneal fluid, lymphatic fluid, pleural fluid, pericardial fluid, cerebrospinal fluid, and in various fluids of the reproductive system. Disease may be caused at any site bathed by these fluids. Other bacteria and viruses reside primarily in different organ systems and in specific tissues, proliferate, and then enter the circulatory system to gain access to other tissues and organs at remote sites.

Infectious organisms, such as viruses, affect billions of people annually. Recent epidemics include the disease known as acquired immune deficiency syndrome (AIDS), believed to be caused by the human immunodeficiency virus (HIV). Related viruses affect other animals such as primates and cats. This virus is rapidly spreading throughout the world and is prevalent in various sub-populations of individuals, including individuals receiving blood transfusions, individuals using contaminated needles, and individuals having contact with infected biological fluids. This disease is also widespread in certain countries and affects more than one-third of the population. No known cure exists. What is needed is a simple, reliable and economic method for reducing the infectivity of the HIV virus so that transmission is decreased. What is also needed is a method to treat biological fluids of infected individuals in order to decrease transmission of the virus to others in contact with these biological fluids. What is also needed is a method to treat blood found in blood banks in order to decrease transmission of the virus through individuals receiving transfusions. Additionally what is needed for HIV as well as other viruses is a mechanism for decreasing the viral load of a human or an animal by treating the plasma of that individual and returning the treated plasma to the individual such that the viral load in the plasma is decreased.

Other major viral infections which affect animals and humans include, but are not limited to, meningitis, cytomegalovirus, and hepatitis in its various forms. While some forms of hepatitis may be treated with

drugs, other forms are not successfully treated and are lethal. At the present time, most anti-viral therapies are directed to preventing or inhibiting viral replication and appear to focus on the initial attachment of the virus to the T4 lymphocyte or macrophage, the transcription of viral RNA to viral DNA and the assembly of new virus during reproduction. However, a major difficulty with existing treatments, especially with regard to HIV, is the high mutation rate of the virus. Many different strains of HIV are resistant or become resistant to anti-viral drug therapy. Furthermore, during anti-viral therapy treatment, resistant strains of the virus may evolve. Finally, many common therapies for HIV infection involve numerous undesirable side effects and require patient compliance with the ingestion of numerous pills every day or several times a day. Unfortunately, many individuals are afflicted with multiple infections caused by more than one infectious organism, such as HIV and hepatitis. Such individuals require even more aggressive and expensive drug regimens to counteract disease progression. Such regimens may cause numerous side effects as well as multi-drug resistance.

Prior art methods of inactivated viruses using chemical agents have relied on organic solvents such as chloroform. However, chloroform denatures many plasma proteins and is unsuitable for use with fluids which will subsequently be administered back to the animal or human. Many of the plasma proteins that are deleteriously affected by chloroform serve important biological functions including coagulation, hormonal responses, and immune responses. Many of these functions are essential to life, and so damage to proteins related to these functions may have an adverse effect on a patient's health, possibly leading to death. Other solvents such as B-propiolactone, detergents such as TWEEN-80, and di- or tri-alkyl phosphates have been used, alone or in combination. Many of these methods, especially those involving detergents, require tedious procedures to ensure removal of the detergent before reintroduction of the

treated plasma sample into the animal or the human. Further, many of the methods described in the prior art involve extensive exposure to elevated temperature in order to kill free virus and infected cells. Numerous proteins contained in biological fluids such as plasma are deleteriously affected by elevated temperatures. Accordingly, what is needed is a method which is simple, effective, does not require the use of elevated temperatures, and does not appreciably denature plasma proteins or extract them from the biological sample being treated.

Prevention of disease and amelioration of the severity of disease caused by infectious organisms is a major goal for modern medicine. Vaccination programs have reduced the occurrence and severity of many diseases although numerous diseases caused by infectious organisms remain without effective vaccines. Accordingly what is needed are new vaccine compositions for providing protection against infectious organisms.

SUMMARY OF THE INVENTION

The present invention solves the problems described above by providing a simple, effective and efficient method for treating fluids containing lipid-containing infectious organisms. The method of the present invention is effective in reducing the concentration of an lipid-containing infectious organisms in a biological fluid. The present invention is also effective in producing a vaccine against the lipid-containing infectious organism by treating a biological fluid containing the infectious organism such that the organism is still present but no longer infectious. A lipid-containing infectious organism, treated in this manner in order to reduce its infectivity, is administered to a recipient, such as an animal or a human, together with a pharmaceutically acceptable carrier and optionally an immunostimulant, in order to provoke an immune response in the

animal or human against antigens from the delipidated infectious organism.

The present invention contemplates treatment of infectious organisms containing a lipid component in their cell wall or envelope. Accordingly, numerous viruses and bacteria are included within the infectious organisms which may be treated with the method of the present invention. The method of the present invention for treating a fluid containing an infectious organism containing lipids comprises: obtaining a fluid containing the infectious organism; contacting the fluid with a first organic solvent capable of solubilizing the lipid in the infectious organism; and, separating a first phase containing the lipids from the infectious organism from a second phase wherein the second phase is substantially free of the lipids and contains reduced levels of the infectious organism. Fluids treated in this manner may optionally be reintroduced into the animal or human.

Fluids which may be treated with the method of the present invention include but are not limited to the following: plasma; serum; lymphatic fluid; cerebrospinal fluid; peritoneal fluid; pleural fluid; pericardial fluid; various fluids of the reproductive system including but not limited to semen, ejaculatory fluids, follicular fluid and amniotic fluid; cell culture reagents such as normal sera, such as fetal calf serum or serum derived from any other animal or human; and immunological reagents such as various preparations of antibodies and cytokines.

Infectious Organisms Treated with the Present Invention

Infectious organisms which may be treated with the method of the present invention include infectious organisms containing lipid. Such infectious organisms include, but are not limited to, viruses and bacteria, provided the virus or bacteria contains lipid in the viral envelope or bacterial cell wall, respectively. The methods of the present invention

reduce infectivity of infectious organisms and also provide vaccines against these organisms.

Viral infectious organisms which may be inactivated by the above system include, but are not limited to, the lipid-containing viruses of the following genuses: *Alphavirus* (alphaviruses), *Rubivurus* (rubella virus), *Flavivirus* (Flaviviruses), *Pestivirus* (mucosal disease viruses), (unnamed, hepatitis C virus), *Coronavirus*, (Coronaviruses), *Torovirus*, (toroviruses), *Arteivirus*, (arteriviruses), *Paramyxovirus*, (Paramyxoviruses), *Rubulavirus* (rubulavriuses), *Morbillivirus* (morbillivuruses), *Pneumovirinae* (the pneumoviruses), *Pneumovirus* (pneumoviruses), *Vesiculovirus* (vesiculoviruses), *Lyssavirus* (lyssaviruses), *Ephemerovirus* (ephemeroviruses), *Cytorhabdovirus* (plant rhabdovirus group A), *Nucleorhabdovirus* (plant rhabdovirus group B), *Filovirus* (filoviruses), *Influenzavirus A, B* (influenza A and B viruses), *Influenza virus C* (influenza C virus), (unnamed, Thogoto-like viruses), *Bunyavirus* (bunyaviruses), *Phlebovirus* (phleboviruses), *Nairovirus* (nairoviruses), *Hantavirus* (hantaviruses), *Tospovirus* (tospoviruses), *Arenavirus* (arenaviruses), unnamed mammalian type B retroviruses, unnamed, mammalian and reptilian type C retroviruses, unnamed, type D retroviruses, *Lentivirus* (lentiviruses), *Spumavirus* (spumaviruses), *Orthohepadnavirus* (hepadnaviruses of mammals), *Avihepadnavirus* (hepadnaviruses of birds), *Simplexvirus* (simplexviruses), *Varicellovirus* (varicelloviruses), *Betaherpesvirinae* (the cytomegaloviruses), *Cytomegalovirus* (cytomegaloviruses), *Muromegalovirus* (murine cytomegaloviruses), *Roseolovirus* (human herpes virus 6), *Gammaherpesvirinae* (the lymphocyte-associated herpes viruses), *Lymphocryptovirus* (Epstein-Bar-like viruses), *Rhadinovirus* (saimiri-ateles-like herpes viruses), *Orthopoxvirus* (orthopoxviruses), *Parapoxvirus* (parapoxviruses), *Avipoxvirus* (fowlpox viruses), *Capripoxvirus* (sheeppoxlike viruses), *Leporipoxvirus* (myxomaviruses), *Suipoxvirus*

(swine-pox viruses), *Molluscipoxvirus* (molluscum contagiosum viruses), *Yatapoxvirus* (yabapox and tanapox viruses), Unnamed, African swine fever-like viruses, *Iridovirus* (small iridescent insect viruses), *Ranavirus* (front iridoviruses), *Lymphocystivirus* (lymphocystis viruses of fish) *Togaviridae, Flaviviridae, Coronaviridae, Enabdoviridae, Filoviridae, Paramyxoviridae, Orthomyxoviridae, Bunyaviridae, Arenaviridae, Retroviridae, Hepadnaviridae, Herpesviridae, Poxviridae,* and any other lipid-containing virus.

These viruses include the following human and animal pathogens: Ross River virus, fever virus, dengue viruses, Murray Valley encephalitis virus, tick-borne encephalitis viruses (including European and far eastern tick-borne encephalitis viruses, hepatitis A virus, hepatitis B virus, hepatitis C virus, human coronaviruses 229-E and OC43 and others (causing the common cold, upper respiratory tract infection, probably pneumonia and possibly gastroenteritis), human parainfluenza viruses 1 and 3, mumps virus, human parainfluenza viruses 2, 4a and 4b, measles virus, human respiratory syncytial virus, rabies virus, Marburg virus, Ebola virus, influenza A viruses and influenza B viruses, *Arenaviruss:* lymphocytic choriomeningitis (LCM) virus; Lassa virus, human immunodeficiency viruses 1 and 2, hepatitis B virus, Vaccinia, Subfamily: human herpes viruses 1 and 2, herpes virus B, Epstein-Barr virus), (smallpox) virus, Yellow fever virus, cowpox virus, poliovirus, Norwalk virus, molluscum contagiosum virus, and any other lipid-containing virus.

Preferred viruses to be treated with the method of the present invention include the various immunodeficiency viruses including but not limited to human (HIV), simian (SIV), feline (FIV), as well as any other form of immunodeficiency virus. Other preferred viruses to be treated with the method of the present invention include but are not limited to hepatitis in its various forms, especially hepatitis A, hepatitis B and hepatitis C. Another preferred virus treated with the method of the present invention

involves the bovine pestivirus. It is to be understood that the present invention is not limited to the viruses provided in the list above. All viruses containing lipid, especially in their viral envelope, are included within the scope of the present invention.

Bacteria constitute another preferred class of infectious organisms which may be treated with the method of the present invention provided the bacteria contains lipid, preferably in its bacterial cell wall. Preferred bacteria to be treated with the method of the present invention include but are not limited to the following: *Staphylococcus; Streptococcus,* including *S. pyogenes; Enterococci; Bacillus, including Bacillus anthracis,* and *Lactobacillus; Listeria; Corynebacterium diphtheriae; Gardnerella* including *G. vaginalis; Nocardia; Streptomyces; Thermoactinomyces vulgaris; Treponema; Camplyobacter; Pseudomonas* including *P.aeruginosa; Legionella; Neisseria* including *N.gonorrhoeae* and *N.meningitides; Flavobacterium* including *F. meningosepticum* and *F. odoratum; Brucella; Bordetella* including *B. pertussis* and *B. bronchiseptica; Escherichia* including *E. coli; Klebsiella; Enterobacter; Serratia* including *S. marcescens* and *S. liquefaciens; Edwardsiella; Proteus* including *P. mirabilis* and *P. vulgaris; Streptobacillus; Rickettsiaceae* including *R. rickettsii; Chlamydia* including *C. psittaci* and *C. trachomatis; Mycobacterium* including *M. tuberculosis, M. intracellulare, M. fortuitum, M. laprae, M. avium, M. bovis, M. africanum, M. kansasii, M. intracellulare,* and *M. lepraemurium;* and *Nocardia*, and any other bacteria containing lipid in their membranes.

Other lipid-containing infectious organisms that may be treated with the method of the present invention include, but are not limited to, protozoa, molds, and fungi.

Accordingly, it is an object of the present invention to provide a method for treating a fluid in order to reduce or eliminate the infectivity of infectious organisms contained therein.

It is another object of the present invention to decrease the concentration of the infectious organism within the fluid.

Yet another object of the present invention is to decrease the infectivity of the infectious organism contained within the fluid.

Still another of the present invention is to use the present method to decrease the concentration and infectivity of infectious organisms contained within a fluid.

It is a specific object of the present invention to decrease the infectivity of infectious organisms contained within a fluid wherein the fluid is plasma.

It is another specific object of the present invention to provide a method to reduce the infectivity and viral load of viruses found within a fluid such as plasma.

Yet another object of the present invention is to completely or partially inactivate and reduce the viral load of viruses contained within a sample such as plasma, wherein the viruses are human immunodeficiency virus, hepatitis in its various forms, or another virus.

Yet another object of the present invention is to reduce the infectivity and concentration of bacteria contained within a fluid, such as plasma.

It is further an object of the present invention to treat infectious organisms with the method of the present invention in order to reduce their infectivity and provide a vaccine comprising a delipidated infectious organism which may be administered to an animal or a human together with a pharmaceutically acceptable carrier and optionally an immunostimulant compound, to prevent or minimize clinical manifestation of disease following exposure to the infectious organism.

It is another specific object of the present invention to provide an anti-viral vaccine.

Yet another object of the present invention is to provide an anti-bacterial vaccine.

It is a further specific object of the present invention to confer immunity to a lipid-containing infectious organism in an animal or human receiving a vaccine comprising a composition comprising an infectious organism treated with the method of the present invention in a pharmaceutically acceptable carrier.

It is another object of the present invention to provide a method useful for development of vaccines against infectious organisms, including but not limited to, viruses and bacteria.

Yet another object of the present invention is to provide a solution containing inactivated viral particles from a treated lipid-containing virus that may be lyophilized and reconstituted when desired for administration to an animal or human.

It is further an object of the present invention to provide a method for treatment of lipid-containing infectious organisms within a fluid which minimizes deleterious effects on proteins contained within the fluid.

It is another object of the present invention to provide a method for reducing the infectivity of lipid-containing infectious organisms, wherein the method does not employ elevated temperatures, chloroform, detergents, or trialkyl phosphates.

These and other objects, advantages, and uses of the present invention will reveal themselves to one of ordinary skill in the art after reading the detailed description of the preferred embodiments and the attached claims.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

By the term "fluid" is meant any fluid containing an infectious organism, including but not limited to, a biological fluid obtained from an

organism such as an animal or human. Such biological fluids obtained from an organism include but are not limited to plasma, serum, cerebrospinal fluid, lymphatic fluid, peritoneal fluid, follicular fluid, amniotic fluid, pleural fluid, pericardial fluid, reproductive fluids and any other fluid contained within the organism. Other fluids may include laboratory samples containing infectious organisms suspended in any chosen fluid. Other fluids include cell culture reagents, many of which include biological compounds such as fluids obtained from living organisms, including but not limited to "normal serum" obtained from various animals and used as growth medium in cell and tissue culture applications.

By the term "first solvent" or "first organic solvent" is meant a solvent, comprising one or more solvents, that facilitates extraction of lipid.

By the term "demulsifying agent" is meant an agent that assists in the removal of the first solvent which may be present in an emulsion in an aqueous layer.

The terms "pharmaceutically acceptable carrier or pharmaceutically acceptable vehicle" are used herein to mean any liquid including but not limited to water or saline, a gel, salve, solvent, diluent, fluid ointment base, liposome, micelle, giant micelle, and the like, which is suitable for use in contact with living animal or human tissue without causing adverse physiological responses, and which does not interact with the other components of the composition in a deleterious manner.

By the term "infectious organism" is meant any lipid-containing infectious organism capable of causing infection. Some infectious organisms include bacteria, viruses, protozoa, parasites, fungi and mold. Some bacteria which may be treated with the method of the present invention include, but are not limited to the following: *Staphylococcus; Streptococcus,* including *S. pyogenes; Enterococci; Bacillus, including Bacillus anthracis,* and *Lactobacillus; Listeria; Corynebacterium diphtheriae; Gardnerella* including *G. vaginalis; Nocardia; Streptomyces;*

Thermoactinomyces vulgaris; Treponema; Camplyobacter; Pseudomonas including *P.aeruginosa; Legionella; Neisseria* including *N.gonorrhoeae* and *N.meningitides; Flavobacterium* including *F. meningosepticum* and *F. odoratum; Brucella; Bordetella* including *B. pertussis* and *B. bronchiseptica; Escherichia* including *E. coli; Klebsiella; Enterobacter; Serratia* including *S. marcescens* and *S. liquefaciens; Edwardsiella; Proteus* including *P. mirabilis* and *P. vulgaris; Streptobacillus; Rickettsiaceae* including *R. rickettsii; Chlamydia* including *C. psittaci* and *C. trachomatis; Mycobacterium* including *M. tuberculosis, M. intracellulare, M. fortuitum, M. laprae, M. avium, M. bovis, M. africanum, M. kansasii, M. intracellulare,* and *M. lepraemurium;* and *Nocardia,* and any other bacteria containing lipid in their membranes.

Viral infectious organisms which may be inactivated by the above system include, but are not limited to the lipid-containing viruses of the following genuses: *Alphavirus* (alphaviruses), *Rubivurus* (rubella virus), *Flavivirus* (Flaviviruses), *Pestivirus* (mucosal disease viruses), (unnamed, hepatitis C virus), *Coronavirus*, (Coronaviruses), *Torovirus*, (toroviruses), *Arteivirus*, (arteriviruses), *Paramyxovirus*, (Paramyxoviruses), *Rubulavirus* (rubulavriuses), *Morbillivirus* (morbillivuruses), *Pneumovirinae* (the pneumoviruses), *Pneumovirus* (pneumoviruses), *Vesiculovirus* (vesiculoviruses), *Lyssavirus* (lyssaviruses), *Ephemerovirus* (ephemeroviruses), *Cytorhabdovirus* (plant rhabdovirus group A), *Nucleorhabdovirus* (plant rhabdovirus group B), *Filovirus* (filoviruses), *Influenzavirus A, B* (influenza A and B viruses), *Influenza virus C* (influenza C virus), (unnamed, Thogoto-like viruses), *Bunyavirus* (bunyaviruses), *Phlebovirus* (phleboviruses), *Nairovirus* (nairoviruses), *Hantavirus* (hantaviruses), *Tospovirus* (tospoviruses), *Arenavirus* (arenaviruses), unnamed mammalian type B retroviruses, unnamed, mammalian and reptilian type C retroviruses, unnamed, type D retroviruses, *Lentivirus* (lentiviruses), *Spumavirus* (spumaviruses),

Orthohepadnavirus (hepadnaviruses of mammals), *Avihepadnavirus* (hepadnaviruses of birds), *Simplexvirus* (simplexviruses), *Varicellovirus* (varicelloviruses), *Betaherpesvirinae* (the cytomegaloviruses), *Cytomegalovirus* (cytomegaloviruses), *Muromegalovirus* (murine cytomegaloviruses), *Roseolovirus* (human herpes virus 6), *Gammaherpesvirinae* (the lymphocyte-associated herpes viruses), *Lymphocryptovirus* (Epstein-Bar-like viruses), *Rhadinovirus* (saimiri-ateles-like herpes viruses), *Orthopoxvirus* (orthopoxviruses), *Parapoxvirus* (parapoxviruses), *Avipoxvirus* (fowlpox viruses), *Capripoxvirus* (sheeppoxlike viruses), *Leporipoxvirus* (myxomaviruses), *Suipoxvirus* (swine-pox viruses), *Molluscipoxvirus* (molluscum contagiosum viruses), *Yatapoxvirus* (yabapox and tanapox viruses), Unnamed, African swine fever-like viruses, *Iridovirus* (small iridescent insect viruses), *Ranavirus* (front iridoviruses), *Lymphocystivirus* (lymphocystis viruses of fish), *Togaviridae, Flaviviridae, Coronaviridae, Enabdoviridae, Filoviridae, Paramyxoviridae, Orthomyxoviridae, Bunyaviridae, Arenaviridae, Retroviridae, Hepadnaviridae, Herpesviridae, Poxviridae,* and any other lipid-containing virus.

These viruses include the following human and animal pathogens: Ross River virus, fever virus, dengue viruses, Murray Valley encephalitis virus, tick-borne encephalitis viruses (including European and far eastern tick-borne encephalitis viruses, hepatitis C virus, human coronaviruses 229-E and OC43 and others (causing the common cold, upper respiratory tract infection, probably pneumonia and possibly gastroenteritis), human parainfluenza viruses 1 and 3, mumps virus, human parainfluenza viruses 2, 4a and 4b, measles virus, human respiratory syncytial virus, rabies virus, Marburg virus, Ebola virus, influenza A viruses and influenza B viruses, *Arenaviruss:* lumphocytic choriomeningitis (LCM) virus; Lassa virus, human immunodeficiency viruses 1 and 2, or any other immunodeficiency virus, hepatitis A virus,

hepatitis B virus, hepatitis C virus, Subfamily: human herpes viruses 1 and 2, herpes virus B, Epstein-Barr virus), (smallpox) virus, cowpox virus, molluscum contagiosum virus.

Solvents for Use in Removal of Lipid from Lipid-Containing Organisms, Especially Infectious Organisms

Numerous organic solvents may be used in the method of the present invention for removal of lipid from lipid-containing organisms, especially infectious organisms, provided that the solvents or combinations thereof are effective in solubilizing lipids. Suitable solvents comprise mixtures of hydrocarbons, ethers, alcohols and amines. Other solvents which may be used with the present invention include amines and mixtures of amines. Preferred solvents are combinations of alcohols and ethers. Another preferred solvent comprises an ether or combinations of ethers. It is preferred that the solvent or combination of solvents has a relatively low boiling point to facilitate removal through a combination of vacuum and possibly heat.

Examples of suitable amines for use in removal of lipid from lipid-containing organisms in the present invention are those which are substantially water immiscible. Typical amines are aliphatic amines having a carbon chain of at least 6 carbon atoms. A non-limiting example of such an amine is $C_6H_{13}NH_2$.

The alcohols which are preferred for use in the present invention, when used alone, include those alcohols which are not appreciably miscible with plasma or other biological fluids. Such alcohols include, but are not limited to, straight chain and branched chain alcohols, including pentanols, hexanols, heptanols, octanols and alcohols containing higher numbers of carbons.

When alcohols are used in combination with another solvent, for example, an ether, a hydrocarbon, an amine, or a combination thereof, C_1-

C_8 containing alcohols may be used. Preferred alcohols for use in combination with another solvent include C_4-C_8 containing alcohols. Accordingly, preferred alcohols that fall within the scope of the present invention are preferably butanols, pentanols, hexanols, heptanols and octanols, and iso forms thereof. Particularly preferred are the butanols (1-butanol and 2-butanol). As stated above, the most preferred alcohol is the C_4 alcohol, butanol. The specific choice of alcohol will depend on the second solvent employed. In a preferred embodiment, lower alcohols are combined with lower ethers.

Ethers, used alone, or in combination with other solvents, preferably alcohols, are another preferred solvent for use in the method of the present invention. Particularly preferred are the C_4-C_8 containing-ethers, including but not limited to, ethyl ether, diethyl ether, and propyl ethers, including but not limited to di-isopropyl ether. Also useful in the present invention are combinations of ethers, such as di-isopropyl ether and diethyl ether. When ethers and alcohols are used in combination as a first solvent for contacting the fluid containing the lipid-containing organism infectious organism, any combination of alcohol and ether may be used provided the combination is effective to partially or completely remove lipid from the infectious organism. In one embodiment lipid is removed from the viral envelope or bacterial cell wall of the infectious organism. When alcohols and ether are combined as a first solvent for treating the infectious organism contained in a fluid, preferred ratios of alcohol to ether in this solvent are about 0.01%-60% alcohol to about 40%-99.99% of ether, with a preferred ratio of about 10%-50% of alcohol with about 50%-90% of ether, with a most preferred ratio of about 20%-45% alcohol and about 55%-80% ether. An especially preferred combination of alcohol and ether is the combination of butanol and di-isopropyl ether. Another especially preferred combination of alcohol and ether is the combination of butanol with diethyl ether. When butanol and di-isopropyl ether are

combined as a first solvent for treating the infectious organism contained in a fluid, preferred ratios of butanol to di-isopropyl ether in this solvent are about 0.01%-60% butanol to about 40%-99.99% of di-isopropyl ether, with a preferred ratio of about 10%-50% of butanol with about 50%-90% of di-isopropyl ether, with a most preferred ratio of about 20%-45% butanol and about 55%-80% di-isopropyl ether. The most preferred ratio of butanol and di-isopropyl ether is about 40% butanol and about 60% di-isopropyl ether.

When butanol is used in combination with diethyl ether in a first solvent, preferred ratios of butanol to diethyl ether in this combination are about 0.01%-60% butanol to about 40%-99.99% of diethyl ether, with a preferred ratio of about 10%-50% of butanol with about 50%-90% of diethyl ether, with a most preferred ratio of about 20%-45% butanol and about 55%-80% diethyl ether. The most preferred ratio of butanol and diethyl ether in a first solvent is about 40% butanol and about 60% diethyl ether. This combination of about 40% butanol and about 60% diethyl ether (vol:vol) has been shown to have no significant effect on a variety of biochemical and hematological blood parameters, as shown for example in U.S. Patent 4,895,558. Further comparisons were made on the serum pH, protein and enzyme activities in human serum when treated with butanol-DIPE (40%-60% V/V). The results are illustrated in the following table.

TABLE 1

		Control	Delipidated
IgA	mg/100ml	168	167
IgM	mg/100ml	144	144
Ceruloplasmin	mg/100ml	1402	1395
Transferrin	mg/100ml	30	31
Albumin	g/100ml	5.12	5.12
Total protein	g/100ml	7.35	7.42
pH		7.37	7.37
GOT	IU	25	23
Alkaline phosphatase	IU	81	80
a-amylase	IU	293	293

This solvent system of butanol-DIPE (40%-60% V/V) does not adversely affect the blood constituents shown in the table above. Also, there appears to be little or no denaturation of plasma proteins or changes in enzyme activity, including the activity of lipid associated enzymes such as lecithin cholesterol acetyltransferase and cholesterol ester transfer protein.

Solvents for Use in Vaccine Production

Different solvents and combinations of solvents may be used for treating an lipid-containing organism, such as an infectious organism, for producing a vaccine using the treated organism. This section describes these solvents and combinations thereof. Suitable solvents comprise hydrocarbons, ethers, alcohols, amines, surfactants, esters and combinations thereof.

Hydrocarbons in their liquid form dissolve compounds of low polarity such as the lipids found in membranes of infectious organisms. Hydrocarbons which are liquid at about 37°C are effective in disrupting a lipid membrane of an infectious organism. Accordingly, hydrocarbons

comprise any substantially water immiscible hydrocarbon which is liquid at about 37°C. Suitable hydrocarbons include, but are not limited to the following: C_5 to C_{20} aliphatic hydrocarbons such as petroleum ether, hexane, heptane, octane; haloaliphatic hydrocarbons such as chloroform, 1,1,2-trichloro-1,2,2-trifluoroethane, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene dichloromethane and carbon tetrachloride, and thioaliphatic hydrocarbons each of which may be linear, branched or cyclic, saturated or unsaturated; aromatic hydrocarbons such as benzene; alkylarenes such as toluene, haloarenes, haloalkylarenes and thioarenes. Other suitable solvents may also include saturated or unsaturated heterocyclic compounds such as pyridine and aliphatic, thio or halo derivatives thereof.

Suitable esters which may be used include, but are not limited to, ethyl acetate, propylacetate, butylacetate and ethylpropionate.

Suitable surfactants which may be used, include but are not limited to the following: sulfates, sulfonates, phosphates (including phospholipids), carboxylates, and sulfosuccinates. Some anionic amphiphilic materials useful with the present invention include but are not limited to the following: sodium dodecyl sulfate (SDS), sodium decyl sulfate, bis-(2-ethylhexyl) sodium sulfosuccinate (AOT), cholesterol sulfate and sodium laurate.

The alcohols which are preferred for use in the present invention, when used alone, include those alcohols which are not appreciably miscible with plasma or other biological fluids. When alcohols are used in combination with another solvent, for example, ether, a hydrocarbon, an amine or a combination thereof, C_1-C_8 containing alcohols may be used. Preferred alcohols for use in combination with another solvent include lower alcohols such as C_4-C_8 containing alcohols. Accordingly, preferred alcohols that fall within the scope of the present invention are preferably butanols, pentanols, hexanols, heptanols and octanols, and iso forms

thereof. Particularly preferred are the butanols (1-butanol and 2-butanol). As stated above, the most preferred alcohol is the C_4 alcohol, butanol. The specific choice of alcohol will depend on the second solvent employed. In a preferred embodiment, lower alcohols are combined with lower ethers.

Ethers, used alone, or in combination with other solvents, preferably alcohols, are another preferred solvent for use in the method of the present invention. Particularly preferred are the C_4-C_8 ethers, including but not limited to, ethyl ether, diethyl ether, and propyl ethers, including but not limited to di-isopropyl ether. Also useful in the present invention are combinations of ethers, such as di-isopropyl ether and diethyl ether.

When ethers and alcohols are used in combination as a first solvent for removing lipid from the infectious organism in order to make a vaccine, any combination of alcohol and ether may be used provided the combination is effective to partially or completely remove lipid from the infectious organism. In one embodiment lipid is removed from the viral envelope or bacterial cell wall of the infectious organism. When alcohols and ether are combined as a first solvent for treating the infectious organism contained in a fluid, preferred ratios of alcohol to ether in this solvent are about 0.01%-60% alcohol to about 40%-99.99% ether, with a preferred ratio of about 10%-50% alcohol with about 50%-90% ether, with a most preferred ratio of about 20%-45% alcohol and about 55%-80% ether. An especially preferred combination of alcohol and ether is the combination of butanol and di-isopropyl ether. Another especially preferred combination of alcohol and ether is the combination of butanol with diethyl ether. When butanol and di-isopropyl ether are combined as a first solvent for treating the infectious organism contained in a fluid, preferred ratios of butanol to di-isopropyl ether in this solvent are about 0.01%-60% butanol to about 40%-99.99% di-isopropyl ether, with a preferred ratio of about 10%-50% butanol with about 50%-90% di-

isopropyl ether, with a most preferred ratio of about 20%-45% butanol and about 55%-80% di-isopropyl ether. The most preferred ratio of butanol and di-isopropyl ether is about 40% butanol and about 60% di-isopropyl ether.

When butanol is used in combination with diethyl ether in a first solvent, preferred ratios of butanol to diethyl ether in this combination are about 0.01%-60% butanol to about 40%-99.99% diethyl ether, with a preferred ratio of about 10%-50% butanol with about 50%-90% diethyl ether, with a most preferred ratio of about 20%-45% butanol and about 55%-80% diethyl ether. The most preferred ratio of butanol and diethyl ether in a first solvent is about 40% butanol and about 60% diethyl ether.

Biological Fluids and Treatment Thereof for Reducing Infectivity of Infectious, Lipid-Containing Organisms

As stated above, various biological fluids may be employed with the method of the present invention in order to reduce the levels or infectivity of the lipid-containing organism in the fluid. In a preferred embodiment of the present invention, plasma obtained from an animal or human is treated with the method of the present invention in order to reduce the concentration and/or infectivity of lipid-containing infectious organisms within the plasma. In this embodiment, plasma may be obtained from an animal or human by withdrawing blood from the animal or human using known methods and treating the blood with conventional methods in order to separate the cellular components of the blood (red and white cells) from the plasma. Such methods are known to one of ordinary skill in the art and include centrifugation and filtration.

Viruses are typically retained in the plasma and are affected by the treatment of the plasma with the method of the present invention. When the lipid-containing organism to be treated is substantially larger than a virus, and may pellet with red and white blood cells under typical

centrifugation conditions for separating cells from plasma, the lipid-containing organism may be separated from the red and white cells using techniques known to one of ordinary skill in the art. Such methods include but are not limited to centrifugation and filtration. One of ordinary skill in the art understands the proper centrifugation conditions for separating such lipid-containing organisms from the red and white cells. Filtration may include diafiltration or filtration through membranes with pore sizes that separate the lipid-containing organism, such as a bacteria from the red cells and white cells. Use of the present invention permits treatment of lipid-containing organisms, such as a bacteria, found within plasma, without deleterious effects on other plasma proteins.

Treatment of lipid-containing organisms in biological fluids other than blood and plasma does not generally involve separation of the cells from the fluid before the delipidation procedure is initiated. For example, follicular fluid and peritoneal fluid may be treated with the present invention to affect the levels and infectivity of lipid-containing organisms without deleterious effects on protein components. The treated fluid may then be returned to an animal or human. Treatment of these non-blood types of fluids affects the lipid-containing organisms in the fluid, including the bacteria and viruses.

Once a biological fluid, such as plasma, is obtained either in this manner, or for example, from a storage facility housing bags of plasma, the plasma is contacted with a first organic solvent as described above which is capable of solubilizing lipid in the lipid-containing infectious organism. The first organic solvent is combined with the plasma in a ratio wherein the first solvent is present in an amount effective to substantially solubilize the lipid in the infectious organism. Preferred ratios of first solvent to plasma (expressed as first organic solvent:plasma) are described in the following ranges: 0.5 - 4.0:0.5 - 4.0; 0.8 - 3.0:0.8 - 3.0; and 1-2:0.8-1.5.